*EDITORIAL*

# Stool DNA methylation assays in colorectal cancer screening

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## Abstract
Colorectal cancer (CRC) is fourth most common cancer in men and third in women worldwide. Developing a diagnostic panel of sensitive and specific biomarkers for the early detection of CRC is recognised as to be crucial for early initial diagnosis, which in turn leads to better long term survival. Most of the research on novel potential CRC biomarkers in the last 2 decades has been focussed on stool DNA analysis. In this paper, we describe the recent advances in non-invasive CRC screening and more specifically in molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions. In several research papers these markers showed superior rates for sensitivity and specificity in comparison to previously described assays. These tests detected the majority of adenomas $\geq$ 1 cm in size and the detection rates progressively increased with larger adenomas. The methylation status of the BMP3 and NDRG4 promoters demonstrated effective detection of neoplasms at all sites throughout the colon and was not affected by common clinical variables. Recently, a multitarget stool DNA test consisting of molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions, mutant KRAS and immunochemical assay for human haemoglobin has been made commercially available and is currently reimbursed in the United States. Although this is the most sensitive non-invasive CRC screening test, there is the need for further research in several areas - establishment of the best timeframe for repeated DNA stool testing; validation of the results in populations outside of North America; usefulness for surveillance and prognosis of patients; cost-effectiveness of DNA stool testing in real-life populations.

Key words: Colorectal cancer; Screening programs; BMP3, NDRG4; Promoter hypermethylation

Core tip: Developing a diagnostic panel of sensitive and specific biomarkers for the early detection of colorectal cancer (CRC) is recognised as to be crucial for early initial diagnosis, which in turn leads to better long term

survival. In this paper, we describe the recent advances in non-invasive CRC screening and more specifically in molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions. These markers show superior rates for sensitivity and specificity in comparison to previously described assays.

## EDITORIAL

Colorectal cancer (CRC) is fourth most common cancer in men and third in women worldwide with highest incidence in developed and newly developed countries and lowest rates in less economically advanced countries such as India[1-3]. Morbidity and mortality associated with colorectal cancer can be reduced by applying lifestyle changes together with targeted screening programs and early therapeutic intervention. CRC screening programs have been adopted in United States[4], Canada[5] and some European countries[6]. While several screening options are provided in the United States[7,8], the most recent European Council directive on CRC screening recommends only faecal occult blood test (FOBT) as initial step offered to individuals aged 50 to 74. Various screening strategies are used in different countries, thus giving the opportunity to assess their cost-effectiveness and explore the possibilities to improve the process[6,9].

The cost-effectiveness of colorectal cancer screening has been analysed in two independent review papers. Based on their findings, CRC screening is clearly cost-effective compared with the "no screening" strategy. However, it is still unclear which screening method is the most effective. The last review concluded that newer modalities such as CT colonography, capsule endoscopy and DNA stool testing were not yet cost-effective compared to the established options (FOBT, colonoscopy, sigmoidoscopy, barium enema)[10,11].

Developing a diagnostic panel of sensitive and specific biomarkers for the early detection of CRC is recognised as to be crucial for early initial diagnosis, which in turn leads to better long term survival[12]. Patients with localised disease (Duckes I and IIA) have about 90% 5-year survival. Flexible colonoscopy is considered to be the gold standard screening option, but it is an invasive procedure, it has the highest complication rates from all screening modalities, it misses about 5% of CRC, and it is relatively expensive. All the available CRC screening modalities appear to be underused due to various reasons, but many patients do not want to undergo colonoscopy in particular due to fear of pain, discomfort or embarrassment[13]. FOBT

used in population-based screening was the first widely available and cheap non-invasive option. However, it requires multiple tests and has suboptimal efficacy[14]. In the last few years, the use of more sensitive and specific methods such as faecal immunochemical test (FIT) or high-sensitivity guaiac-based faecal occult blood testing (hsFOBT) has been favoured by guidelines and panel recommendations[8,15-17].

The matter of potential patient acceptance and interest in a hypothetical multiorgan stool-DNA test (MUST) for pan-digestive cancer screening was studied recently[18]. The authors designed a special 29-item survey questionnaire related to demographics, knowledge of digestive cancers, personal and family history of cancer, personal concern of cancer, CRC screening behaviour, interest in MUST, importance of test features in a cancer screening tool, and comparison of MUST with available CRC screening tests. The questionnaire was mailed to 1200 randomly-selected patients from the Mayo Clinic registry. The survey was completed by 434 participants. MUST was preferred over other tests by 98% of the responders. In general, non-invasive tests (MUST, colorectal-only stool-DNA testing, and FOBT) were preferred over the invasive tests (colonoscopy, flexible sigmoidoscopy, and barium enema). Patients reported that they preferred MUST, because there was no need for bowel preparation and sedation, no risk of complications and due to satisfactory test accuracy. Hence, identification of novel biomarkers that are simple, non-invasive, cost-efficient, and highly specific and sensitive and their implementation into clinical practice would be of great clinical benefit[19].

Ideally, the potential biomarker should be sensitive for detection of adenomas and allow early CRC diagnosis. Furthermore, the marker should be chemically stable to allow transportation and not to be affected by clinical variables such as patients' race, ethnicity, age, chronic disorders, habits or environmental factors. It would be of additional benefit if the potential biomarker had some prognostic value and allowed surveillance after surgery. Most importantly, the test results should allow simple interpretation, thus ensuring smooth translation into clinical practice.

Most of the research on novel potential CRC biomarkers in the last 2 decades has been focussed on stool (faecal) DNA analysis[20]. There have been three major genetic mechanisms identified to be involved in early CRC and precancerous colorectal lesions: (1) chromosomal instability due to mutations in APC, KRAS and TP53; (2) microsatellite instability due to loss of function in mismatch repair genes; and (3) DNA methylation, which is an epigenetic alteration leading to promotor hypermethylation and subsequent suppression of gene transcription[20]. The testing strategy has gradually evolved, with KRAS initially targeted as a single marker in the 1990s. Later, a lot of research was conducted on Wnt

signalling pathway and microsatellite instability, but it was not as successful as expected. Lately, most of the research has been focussed on methylated genes such as vimentin, TFPI2 and NDRG4, BMP3 and SFRP2[21,22].

The role of NDRG4 as tumour suppressor gene was first addressed in 2009 by Melotte *et al*[23]. They studied NDRG4 promoter methylation in human colorectal cancer cell lines, colorectal tissue and normal colonic mucosa. Methylation of the promoter region occurred in 70%-86% of CRC tissues compared with 4% in non-cancerous colonic mucosa. In stool DNA, methylated NDRG4 showed a sensitivity of 61% and 53% for detecting CRC in training and validation sets, respectively, with corresponding specificities of 93% and 100%[23].

The first evidence for the importance of BMP3 inactivation in early polyp formation and colorectal tumour development has been first published by Kim Loh in 2008. The authors observed aberrant BMP3 hypermethylation in 33/60 (55%) tumours. They noticed that this event was highly correlated with microsatellite instability ($P < 0.01$), the CpG Island Methylator Phenotype ($P < 0.01$), BRAF oncogene mutation ($P < 0.01$), and proximal tumour location ($P < 0.001$). Their observation suggested that BMP3 is an attractive target for the future development of molecular blood and/or stool screening tests for the early detection of lesions with neoplastic potential[24].

A small study investigating stool DNA testing of BMP3 and NDRG4 hypermethylation for monitoring after CRC resection has been published recently. The authors demonstrated that methylated gene markers in the stool samples from 14 patients with CRC and elevated pre-operative levels decrease after surgery unless disease is still present or recurring[25].

In 2012, Ahlquist *et al*[26] performed a blind, multicentre, case-control study using stool samples from 252 patients with CRC, 133 with adenomas ⩾ 1 cm, and 293 individuals with normal colonoscopy results (controls). Their study included four methylated genes (vimentin, NDRG4, BMP3, and TFPI2), mutant KRAS, a reference gene β-actin, and haemoglobin. By the use of this panel, they identified 85% of patients with CRC and 54% of patients with adenomas ⩾ 1 cm in size with 90% specificity. One of the important findings of the study was that the DNA test detected the majority of adenomas ⩾ 1 cm and the detection rates progressively increased with larger adenomas. Other important finding was that the test showed effective detection of neoplasms at all sites throughout the colon. In comparison to other routine screening methods, this test showed higher performance and sensitivity rates for proximal colon neoplasms[26]. In another study, Ahlquist *et al*[27] demonstrated the effect of common clinical variables on the same four candidate methylation markers. The methylation levels of all four markers increased with age, $P < 0.0001$. The relative increase per standard deviation of age

was greatest with TEPI2 at 49.4% and least with BMP3 at 0.21%. The four markers were not affected by smoking, alcohol consumption, NSAIDs, personal or family history of colorectal neoplasia or body mass index variations. It could be concluded that only the least affected by clinical variables markers could be recommended for further studies and proposed for the CRC screening.

Eventually, in a large study published in 2014, Imperiale compared a multi-target stool DNA test with a commercial FIT among a large number of asymptomatic subjects at average-risk for CRC ($n$ = 9989). The authors simulated a real life screening scenario and applied powerful statistical tools to evaluate the true performance of the test used. The multitarget stool DNA test consisted of molecular assays for aberrantly methylated BMP3 and NDRG4 promoter regions, mutant KRAS, and β-actin (a reference gene for human DNA quantity), as well as an immunochemical assay for human haemoglobin. The sensitivity of the DNA test for the detection of CRC reached 92.3% and 42.4% for advanced precancerous lesions. FIT screening test detected 74% of cancers and 24% of advanced adenomas. The DNA test showed higher sensitivity, while FIT was more specific and had higher rate of successfully processed samples[28,29].

Subsequently, the test was approved by FDA in August 2014, has been made commercially available as Cologuard™ and is currently reimbursed by Medicare in the United States as part of the CRC screening under the Affordable Care Act. Cologuard™ has been approved for screening of average risk, asymptomatic individuals aged 50-85 and Medicare pays for the test to be done every 3 years[30]. This is the first commercially available DNA stool test to become a part of a national screening programme. At present, there is insufficient data to comment whether the 3 year period for repeated testing is adequate or not.

## CONCLUSION

Recent advances have made it possible for a combined FIT and multitarget DNA stool test to become commercially available and reimbursed as a part of the CRC screening in the United States. The approval was based on the results of a large cross-sectional study in North America. The statistical analysis of the clinical data was consistent with significantly increased sensitivity (nearly 20% in absolute terms) at the cost of somewhat decreased specificity compared to FIT alone. Up to date this is the most sensitive non-invasive CRC screening test available. However, further research seems to be needed in several areas: (1) to establish the best timeframe for repeated DNA stool testing; (2) to validate the results in populations outside of North America; (3) to establish if any of the DNA stool markers can be used for the surveillance of

patients after a CRC surgery with curative intent; and (4) to determine how cost-effective is DNA stool testing in real-life populations. Moreover, further DNA stool test panels are likely to become commercially available in the next years and will need to be clinically validated.

## REFERENCES

1   **Center MM**, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. *CA Cancer J Clin* 2009; **59**: 366-378 [PMID: 19897840 DOI: 10.3322/caac.20038]

2   **Siegel R**, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 104-117 [PMID: 24639052 DOI: 10.3322/caac.21220]

3   **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]

4   **Green BB**, Coronado GD, Devoe JE, Allison J. Navigating the murky waters of colorectal cancer screening and health reform. *Am J Public Health* 2014; **104**: 982-986 [PMID: 24825195 DOI: 10.2105/AJPH.2014.301877]

5   **Honein-AbouHaidar GN**, Rabeneck L, Paszat LF, Sutradhar R, Tinmouth J, Baxter NN. Evaluating the impact of public health initiatives on trends in fecal occult blood test participation in Ontario. *BMC Cancer* 2014; **14**: 537 [PMID: 25062552 DOI: 10.1186/1471-2407-14-537]

6   **Altobelli E**, Lattanzi A, Paduano R, Varassi G, di Orio F. Colorectal cancer prevention in Europe: burden of disease and status of screening programs. *Prev Med* 2014; **62**: 132-141 [PMID: 24530610 DOI: 10.1016/j.ypmed.2014.02.010]

7   **Grobbee EJ**, Kapidzic A, van Vuuren AJ, van Leerdam M, Lansdorp-Vogelaar I, Looman CW, Bruno MJ, Kuipers EJ, Spaander MC. Second-Look Colonoscopies and the Impact on Capacity in FIT-Based Colorectal Cancer Screening. *Am J Gastroenterol* 2015; **110**: 1072-1077 [PMID: 26054624 DOI: 10.1038/ajg.2015.157]

8   **Levin B**, Lieberman DA, McFarland B, Andrews KS, Brooks D, Bond J, Dash C, Giardiello FM, Glick S, Johnson D, Johnson CD, Levin TR, Pickhardt PJ, Rex DK, Smith RA, Thorson A, Winawer SJ. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *Gastroenterology* 2008; **134**: 1570-1595 [PMID: 18384785 DOI: 10.1053/j.gastro.2008.02.002]

9   **Pawa N**, Arulampalam T, Norton JD. Screening for colorectal cancer: established and emerging modalities. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 711-722 [PMID: 22045159 DOI: 10.1038/nrgastro.2011.205]

10  **Pignone M**, Saha S, Hoerger T, Mandelblatt J. Cost-effectiveness analyses of colorectal cancer screening: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002; **137**: 96-104 [PMID: 12118964 DOI: 10.7326/0003-4819-137-2-200207160-00007]

11  **Lansdorp-Vogelaar I**, Knudsen AB, Brenner H. Cost-effectiveness of colorectal cancer screening. *Epidemiol Rev* 2011; **33**: 88-100 [PMID: 21633092 DOI: 10.1093/epirev/mxr004]

12  **Schreuders EH**, Ruco A, Rabeneck L, Schoen RE, Sung JJ, Young GP, Kuipers EJ. Colorectal cancer screening: a global overview of existing programmes. *Gut* 2015; Epub ahead of print [PMID: 26041752 DOI: 10.1136/gutjnl-2014-309086]

13  **McLachlan SA**, Clements A, Austoker J. Patients' experiences and reported barriers to colonoscopy in the screening context--a systematic review of the literature. *Patient Educ Couns* 2012; **86**: 137-146 [PMID: 21640543 DOI: 10.1016/j.pec.2011.04.010]

14  **Fraser CG**. Faecal occult blood tests--eliminate, enhance or update? *Ann Clin Biochem* 2008; **45**: 117-121 [PMID: 18325172 DOI: 10.1258/acb.2008.007223]

15  **Duffy MJ**, van Rossum LG, van Turenhout ST, Malminiemi O, Sturgeon C, Lamerz R, Nicolini A, Haglund C, Holubec L, Fraser CG, Halloran SP. Use of faecal markers in screening for colorectal neoplasia: a European group on tumor markers position paper. *Int J Cancer* 2011; **128**: 3-11 [PMID: 20824704 DOI: 10.1002/ijc.25654]

16  **Rex DK**, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *Am J Gastroenterol* 2009; **104**: 739-750 [PMID: 19240699 DOI: 10.1038/ajg.2009.104]

17  **Stracci F**, Zorzi M, Grazzini G. Colorectal cancer screening: tests, strategies, and perspectives. *Front Public Health* 2014; **2**: 210 [PMID: 25386553 DOI: 10.3389/fpubh.2014.00210]

18  **Yang D**, Hillman SL, Harris AM, Sinicrope PS, Devens ME, Ahlquist DA. Patient perceptions of stool DNA testing for pan-digestive cancer screening: a survey questionnaire. *World J Gastroenterol* 2014; **20**: 4972-4979 [PMID: 24803808 DOI: 10.3748/wjg.v20.i17.4972]

19  **Lidgard GP**, Domanico MJ, Bruinsma JJ, Light J, Gagrat ZD, Oldham-Haltom RL, Fourrier KD, Allawi H, Yab TC, Taylor WR, Simonson JA, Devens M, Heigh RI, Ahlquist DA, Berger BM. Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clin Gastroenterol Hepatol* 2013; **11**: 1313-1318 [PMID: 23639600 DOI: 10.1016/j.cgh.2013.04.023]

20  **Goel A**, Boland CR. Epigenetics of colorectal cancer. *Gastroenterology* 2012; **143**: 1442-1460.e1 [PMID: 23000599 DOI: 10.1053/j.gastro.2012.09.032]

21  **Wang X**, Kuang YY, Hu XT. Advances in epigenetic biomarker research in colorectal cancer. *World J Gastroenterol* 2014; **20**: 4276-4287 [PMID: 24764665 DOI: 10.3748/wjg.v20.i15.4276]

22  **Zhang H**, Qi J, Wu YQ, Zhang P, Jiang J, Wang QX, Zhu YQ. Accuracy of early detection of colorectal tumours by stool methylation markers: a meta-analysis. *World J Gastroenterol* 2014; **20**: 14040-14050 [PMID: 25320544 DOI: 10.3748/wjg.v20.i38.14040]

23  **Melotte V**, Lentjes MH, van den Bosch SM, Hellebrekers DM, de Hoon JP, Wouters KA, Daenen KL, Partouns-Hendriks IE, Stessels F, Louwagie J, Smits KM, Weijenberg MP, Sanduleanu S, Khalid-de Bakker CA, Oort FA, Meijer GA, Jonkers DM, Herman JG, de Bruïne AP, van Engeland M. N-Myc downstream-regulated gene 4 (NDRG4): a candidate tumor suppressor gene and potential biomarker for colorectal cancer. *J Natl Cancer Inst* 2009; **101**: 916-927 [PMID: 19535783 DOI: 10.1093/jnci/djp131]

24  **Loh K**, Chia JA, Greco S, Cozzi SJ, Buttenshaw RL, Bond CE, Simms LA, Pike T, Young JP, Jass JR, Spring KJ, Leggett BA, Whitehall VL. Bone morphogenic protein 3 inactivation is an early and frequent event in colorectal cancer development. *Genes Chromosomes Cancer* 2008; **47**: 449-460 [PMID: 18311777 DOI: 10.1002/gcc.20552]

25  **Kisiel JB**, Yab TC, Taylor WR, Mahoney DW, Ahlquist DA. Stool methylated DNA markers decrease following colorectal cancer resection--implications for surveillance. *Dig Dis Sci* 2014; **59**: 1764-1767 [PMID: 24993691 DOI: 10.1007/s10620-014-3265-5]

26  **Ahlquist DA**, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszat LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sørensen HT, Berger BM, Lidgard GP. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 2012; **142**: 248-256; quiz e25-e26 [PMID: 22062357 DOI: 10.1053/j.gastro.2011.10.031]

27  **Ahlquist DA**, Taylor WR, Yab TC, Devens ME, Mahoney DW, Boardman LA, Thibodeau SN, Hongzhi Z, Domanico MJ, Berger BM and Lidgard GP. Aberrantly Methylated Gene Marker Levels in Stool: Effects of Demographic, Exposure, Body Mass, and Other Patient Characteristics. *J Mol Biomark Diagn* 2012; **3**: 133 [DOI: 10.4172/2155-9929.1000133]

28  **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; **351**: 2704-2714 [PMID: 15616205 DOI: 10.1056/NEJMoa033403]

29  **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM. Multitarget stool DNA

testing for colorectal-cancer screening. *N Engl J Med* 2014; **370**: 1287-1297 [PMID: 24645800 DOI: 10.1056/NEJMoa1311194]

30    **Hwang TJ**, Lehmann LS, Kesselheim AS. Precision medicine and the FDA's draft guidance on laboratory-developed tests. *Nat Biotechnol* 2015; **33**: 449-451 [PMID: 25965745 DOI: 10.1038/nbt.3221]

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